

### **Instrument Operating Procedure**

- I. **PURPOSE:** To describe procedures to be followed for the use, inspection, cleaning, calibration/standardization, testing, and maintenance of:

- A. **Instrument:** Leica AutoStainer XL Automated Slide Stainer
- B. **Manufacturer:** Leica Microsystems Nussloch GmbH, Heidelberger Str. 17  
- 19, D-69226 Nussloch, Germany
- C. **Model:** AutoStainer XL
- D. **Serial No:** ST5010
- E. **Location:** Bozeman Fish Technology Center, Laboratory #2

**Note:** This SOP is written specifically for use when conducting non-clinical studies in support of new animal drug approvals. If used during other studies, or for other purposes, appropriate procedures and programs will be used, and the instrument logs will be maintained. A signature/initial card will be maintained in AADAP Program files.

II. **INSPECTION:**

- A. Before use, check to make sure that:
  - 1. The AutoStainer is on a stable bench.
  - 2. If the AutoStainer is not already ON, turn the power on by using the red switch on the right-hand side.
  - 3. All reagent levels have been inspected. See Appendix A for stations and changing schedule for reagents.
  - 4. Water at sink is turned ON.

III. **TESTING**

- A. No testing is necessary before use.

IV. **CALIBRATION:**

- A. No calibration is necessary before use.

V. **USE:** (Refer to Instruction Manual, pages 3 - 50 for more detailed instructions.)

**Note:** Only qualified personnel that have been trained in the use of the Leica AutoStainer XL by Ms. Molly Bowman, Dr. Molly Webb, or other designee may use the Leica AutoStainer XL.

A. Staining:

1. To begin staining, press STAIN (F1) on the control panel.
2. Choose the appropriate program (**Note:** Select **Program 5** for all fish tissue samples processed for aquaculture drug efficacy or target animal safety studies) by using the up and down arrows, and check to make sure the LOAD button light is ON.
3. The machine will take a few seconds to initialize. When it has finished, open the Load drawer, and place the slide holder with slides in the drawer. Close the Load drawer, and press the LOAD button; staining will begin.
  - a. A slide holder with slides can be placed into the Load drawer for staining whenever the LOAD button light is ON.
4. When staining is finished, the EXIT button light will be ON, and a beeper will sound every 30 seconds. Open the Exit drawer slowly, remove the slide rack, close the Exit drawer, and then press the EXIT button. **Note:** If the EXIT button is not pressed, the instrument will be unable to finish the processing of additional racks.
  - a. When the final step in a program is not the Exit drawer, the UNLOAD button on the control panel will flash when processing is complete.
    1. Press UNLOAD (F1) on the control panel, and the station number of the completed rack will be displayed.
    2. If the station number is correct, press UNLOAD (F1) on the control panel, open the lid, and remove the rack.

3. Press DONE (F1) on the control panel. **Note:** If the DONE button is not pressed, the instrument will be unable to finish the processing of additional racks.

B. Interrupting staining:

1. To interrupt staining press the PAUSE (F4) button on the control screen.
  - a. Press STAIN (F1) to continue staining.

C. Programming:

1. To enter a new program, refer to the Instruction Manual, pages 22 - 28.

VI. **MAINTENANCE and CLEANING:**

A. Routine:

1. Clean interior stainless steel surfaces with detergent, and rinse with water. Clean the head covers by wiping with a damp cloth.
2. Reagent and wash containers can be removed and washed with detergent and warm water. **Note:** Do not wash the reagent or wash containers in an automatic dishwasher.
3. Periodically check the wax tray at the bottom of the oven; clean if excessive wax dripping has occurred.

B. Service agreement:

1. No service agreement is currently in place.

VII. **MALFUNCTIONS and REPAIR:**

- A. In case of a malfunction, refer to the Instruction Manual (pages 39 - 43) or call the Leica Technical Assistance Center, 1-800-248-0123. Describe the problem and any corrective action taken on the Instrument/Equipment log.

**VIII. PERSONS RESPONSIBLE FOR EACH OPERATION:**

- A. Persons responsible for operating the Leica AutoStainer will be properly trained in its use.
- B. Training and experience will be reflected in the user's Curriculum Vitae.

**APPROVED BY:** \_\_\_\_\_ **DATE** \_\_\_\_\_  
Management

**APPROVED BY:** \_\_\_\_\_ **DATE** \_\_\_\_\_  
Study Director

**Table 1.** Program 5 of the Leica XL AutoStainer.

## PROGRAM 5

Step Number	Station Number	Reagent	Time in Reagent
1	Oven	60°C	35 min
2	1	Pro-Par	5 min
3	2	Pro-Par	5 min
4	3	100% ETOH	5 min
5	4	100% ETOH	5 min
6	5	95% ETOH	5 min
7	Wash 1	Water	30 sec
8	6	Hematoxylin	4 min
9	Wash 2	Water	8 min
10	7	Acid Alcohol	45 sec
11	Wash 3	Water	45 sec
12	8	Scott's Solution	1 min
13	9	80% ETOH	30 sec
14	10	Eosin - Y	1.5 min
15	11	80% ETOH	1 min
16	12	95% ETOH	2 min
17	13	100% ETOH	2 min
18	14	Pro-Par	3 min
19	15	Pro-Par	3 min
20	EXIT Drawer (approximately 1 hr 28 min) Remove Slide Rack and Press EXIT Drawer button		

Recipes for Acid alcohol, Scott's working solution, and Scott's stock solution as used in the Hematoxylin and Eosin stain are as follows:

Acid alcohol:  
12 mL Acetic acid  
432 mL 70% ETOH

Scott's solution:  
385 mL distilled water  
59 mL Scott's stock solution

Scott's stock solution:  
1 g potassium bicarbonate  
10 g magnesium sulphate  
500 mL distilled water

SOP No. INST 137

Instrument/Equipment Inspection, Testing, and Maintenance Log<sup>1</sup>

Instrument/Equipment: Leica XL AutoStainer

Model #: XL  
ST5010

Serial #:

Initials	Date	Inspection	Calibration	Testing	Maintenance	Follow SOP?	Comments / Number of Slide Racks

<sup>1</sup>Make an entry in each space: (a) If not required, enter NR; (b) If required, but not done, explain.

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Study Director \_\_\_\_\_